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Genetics and cytology of a new genic male-sterile soybean [Glycine max (L.) Merr.]

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Keywords
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Introduction

Male sterility is a condition in plants in which male gametophytic function is prevented, but the potential for female reproduction remains. Based on inheritance patterns, there are two general types of male sterility: genic (nuclear) male sterility (gms) and cytoplasmic male sterility (cms). Male-sterile mutations provide source material for studies in plant breeding, genetics, reproductive biology, and molecular biology. Male sterility has been used in soybean breeding studies (Brim and Stuber 1973; Lewers et al. 1996), but so far it has not been used for commercial production of hybrid seed because large quantities of hybrid seed cannot be produced at the present time. During the past two decades, six genic male-sterile mutations (*ms1*, *ms2*, *ms3*, *ms4*, *ms5*, and *ms6*) have been reported in soybean (Palmer et al. 1992). All of these are nuclear mutations inherited as monogenic recessive traits. Cms has not been confirmed in soybean.

Observations on the genetics and developmental reproductive biology of most of the soybean mutants have been summarized (Graybosch and Palmer 1988; Palmer et al. 1992). In soybean mutants *ms2* and *ms3*, male sterility is due to abortion of microspores caused by failure of callose dissolution at the tetrad stage. In the present study, we observed a similar phenomenon leading to microspore abortion in a potentially new male-sterile line. Therefore, the objectives of this study were to determine whether this soybean mutant was a cytoplasmic male-sterile or a new genic male-sterile line with phenotypic characteristics different from *ms1*, *ms4*, and *ms6*, but similar to *ms2* and *ms3*, and to determine the extent of

callase activity in anthers from male-fertile and male-sterile plants (see Table1).

Materials and methods

Genetics

Seeds of the male-sterile line were obtained from Midwest Oilseeds, Adel, Iowa. This line has unusually high seed set in the field. To test the completeness of male sterility, 300 plants, which were progeny of known heterozygotes, were grown in a glasshouse at Iowa State University, Ames, in the summer of 1995, in the absence of insect pollinators. At anthesis, plants were classified for fertility and sterility based on the presence or absence of pollen. Fertile plants were rogued. The male-sterile plants were saved and checked for seed set a month after the plants had completed flowering.

Inheritance studies were conducted to determine whether the mutation was a cytoplasmic male-sterile mutant or whether the mutation was nuclear. Allelism tests also were conducted to determine whether the new mutation arose at a new locus, or represented an independent mutation at one of the previously described loci (*ms1*, *ms2*, *ms3*, *ms4*, *ms5*, or *ms6*) (Table 1). Crosses were conducted in 1993 by using a known recessive sterile homozygote as the female parent and the F_1 hybrid (heterozygote) from Midwest Oilseeds as the male parent. F_1 seeds were planted either in a glasshouse at Iowa State University, Ames, or at the University of Puerto Rico Soybean Breeding Nursery, Isabela Substation, Isabela, Puerto Rico. F_1 plants were single-plant threshed and the F_2 seeds were planted at the Bruner Farm near Ames, Iowa. For certain cross combinations, fertile F_2 plants were single-plant threshed and the F_3 seeds were planted in Puerto Rico. All F_1, F_2, and F_3 plants were classified for male sterility/fertility at maturity.

Male gamete transmission tests were conducted with F_1 hybrids from Midwest Oilseeds as the male parent. Cross pollinations were made by using five plant introduction (PI) lines (PI 91167, PI 261474, PI 427099, PI 297544, PI 227333) and a cultivar, A.K. Harrow, as the female parents.

Linkage tests were conducted between the male-sterile mutant and the flower color (*W1*) locus, and between the male-sterile mutant and the pubescence color (*T1*) locus. Linkage determinations are presented by using the general relationship a=XY, b=Xy, c=xY, and d=xy (Skorupska and Palmer 1989) for gene pairs listed as Xx and Yy. Plants were classified as having either purple or white flowers at flowering, and as having either tawny or gray pubescence at maturity.

Microscopy

Cytological observations of anther and pollen development were obtained by collecting reproductive buds of various sizes from both male-fertile and male-sterile plants. Male-fertile and male-sterile plants were identified by squashing late-stage anthers in an aqueous solution of I_2KI (Jensen 1962); anthers from male-fertile plants displayed densely staining pollen grains, whereas anthers from male-sterile plants were almost empty, with only a few stained bodies. Buds from both lines were dissected, and individual anthers were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.24, for 14–16 h at 4°C. After three buffer washes, anthers were postfixed in 1% osmium tetroxide for 1 h at room temperature in the same buffer, washed again with the buffer, dehydrated in a graded acetone series, embedded in Spurr's resin (hard recipe), and polymerized at 70°C for 24 h.

Specimen blocks were sectioned on a Reichert Ultracut S microtome. For light microscopic observations, 1-µm-thick sections were stained with methylene blue-azure II and basic fuchsin (Humphrey and Pittman 1974). Specimens were observed and photographed on a Leitz orthoplan microscope.

For fluorescence microscopy, buds were fixed in a 3:1 mixture of ethanol:glacial acetic acid. Anthers were removed and squashed in a 0.005% solution of aniline blue in 0.15 M phosphate buffer, pH 8.2 (Jensen 1962), to detect the presence or absence of callose.

In vitro enzyme activity assay

For in vitro callase activity assay, flowers of known developmental stages from fertile and sterile plants were chosen. Crude callase was extracted from male-fertile and male-sterile anthers at the tetrad stage according to Frankel et al. (1969), with modifications. The anthers were removed from the flower buds and placed in 1.5-ml microfuge tubes chilled on dry ice. The anthers were ground and treated with extraction buffer consisting of 0.08 M acetate and 0.08 M NaCl, pH 4.8, and then allowed to sit for 30 min at room temperature; the tubes were then microfuged for 15 min at 4°C. The supernatant contained the crude enzyme extract. Isolated tetrads from fertile and sterile anthers were placed on separate glass slides, the enzyme extracts were added, and the preparations were coverslipped. The four combinations were: (1) fertile anther extract to fertile tetrads, (2) fertile anther extract to sterile tetrads, (3) sterile anther extract to fertile tetrads and (4) sterile anther extract to sterile tetrads. The reaction mixtures were incubated for about 18 h at 37°C in a moist chamber. After incubation, lacmoid (0.1% resorcin blue in absolute ethanol) was added to stop the reaction and to stain the undigested callose (Frankel et al. 1969). Color intensity and presence of tetrad callose walls were determined and recorded photographically on a Leitz orthoplan microscope.

Table 1 Phenotypic expression of genic male-sterile, female-fertile mutants in soybean. Mutant *ms5 ms5* has not been studied cytologically; ms ms is the mutant described in this study (seeds from Midwest Oilseeds)

Mutant	Meiocyte	Tetrad	Microspore	Pollen
ms1 ms1	——	Failure cytokinesis, tapetum degeneration	——	——
ms2 ms2	——	Callose retention, no microspore wall formed, tapetum degeneration	——	——
ms3 ms3	——	Callose retention, microspore wall initiated, tapetum degeneration	——	——
ms4 ms4	——	Failure cytokinesis, tapetum degeneration	——	——
ms6 ms6	——	Tapetum degeneration	——	——
msp msp	Inconsistent, abortion occurs between premeiocyte and pollen stages			
ms ms	——	Callose retention, microspore wall initiated	——	——

Results

Genetics

In the glasshouse experiment without the presence of insect pollinators, there were no pods formed on the male-sterile plants, which indicated that this was a completely male-sterile line and that the summer glasshouse environment (June to August 1995) did not influence the expression of the male-sterility gene. Classifications of F_2 plants obtained from cross-pollination in the male gamete transmission test are given in Tables 2 and 3. Table 2 shows that the ratio of nonsegregating and segregating families is 1:1, indicating that the two male gametes transmitted equally. A ratio of 3 fertile plants:1 sterile plant in segregating F_2 families was observed (Table 3), which indicates that male sterility is conditioned by a single-recessive gene. Therefore, this genic male-sterile mutant will be designated as ms ms and its fertile heterozygote as Ms ms.

In linkage tests between the ms and *W1* (flower color) loci, χ^2=2.65 was calculated with *P*=0.45 (Table 4), and for independent assortment between the ms and *T1* (pubescence color) loci, χ^2=0.30 and *P*=0.96 (Table 5). In both tests, the observed values fit the expected ratios of 9:3:3:1. There was no linkage between the ms and *W1* loci, or between the ms and *T1* loci.

Classifications of F_2 families obtained from allelism tests are given in Tables 6 and 7. If ms was a mutation at a different locus than the one tested, the F_1 populations would contain only male-fertile plants. In the F_2, 50% of the F_1-derived families would segregate in a ratio of 3 male-fertile plants:1 male-sterile plant, and 50% would produce a population consisting of 9 male-fertile plants:7 male-sterile plants. No male-sterile plants were observed in any of the F_1 generations (data not shown). This indicates that the allele for male sterility in this genic male-sterile mutant was at a locus different from the six known genic male-sterile loci. In the F_2 generation, there were two kinds of families with almost equal frequency, except for *ms3* and *ms4*. These families segregated in 3:1

Table 2 Male gametophyte transmission test: F_2 data

Cross combinations	Number of families		χ^2 (1:1)	*P* (*df*=1)
	Nonsegregating	Segregating		
PI 91167×M2[a]	9	17	2.46	0.12
PI 261474×M2	10	8	0.22	0.64
PI 427099×M2	7	6	0.08	0.78
PI 297544×M2	12	6	2.00	0.16
PI 227333×M2	8	9	0.06	0.81
A. K. Harrow×M2	6	5	0.09	0.76
Total	52	51	0.01	0.92

[a] Midwest Oilseeds M2=502–1×71005–7; F_1 plants are heterozygous, Ms ms

Table 3 Segregation for fertility/sterility in segregating F_2 families

Cross combinations	Number of plants		χ^2(3: 1)	*P* (*df*=1)
	Fertile	Sterile		
PI 91167×M2[a]	978	306	0.93	0.33
PI 261474×M2	505	161	0.24	0.62
PI 427099×M2	379	136	0.55	0.46
PI 297544×M2	479	159	0.00	1.00
PI 227333×M2	747	249	0.00	1.00
A.K. Harrow×M2	449	129	2.21	0.14
Total	3537	1140	0.97	0.32

[a] Midwest Oilseeds M2=502–1×71005–7; F_1 plants are heterozygous, Ms ms

Table 4 Linkage test between ms and *W1* loci in soybean: F_2 data. Linkage determinations: a=XY, b=Xy, c=xY, d=xy

Cross combinations	Frequency of phenotype				Total	χ^2 (9:3:3:1)	*P* (*df*=3)
	a	b	c	d			
PI 91167×M3[a]	140	44	46	17	247	0.24	0.97
PI 261474×M3	42	18	12	7	79	0.32	0.96
PI 427099×M1[b]	97	28	40	10	175	0.12	0.99
PI 297544×M1	210	82	60	35	387	2.61	0.46
PI 227333×M3	182	52	48	19	301	0.92	0.82
A.K. Harrow×M3	188	54	52	16	310	0.05	1.00
Total	859	278	258	104	1499	2.65	0.45

[a] Midwest Oilseeds M3=527–8×91133; F_1 plants are heterozygous, Ms ms
[b] Midwest Oilseeds M1=502–19×82854; F_1 plants are heterozygous, Ms ms

Table 5 Linkage test between ms and *T1* loci in soybean: F_2 data. Linkage determinations: a=XY, b=Xy, c=xY, d=xy

Cross combinations	Frequency of phenotype				Total	χ^2 (9:3:3:1)	*P* (*df*=3)
	a	b	c	d			
PI 91167×M3[a]	97	30	27	6	160	0.44	0.93
PI 261474×M3	127	48	35	10	220	0.50	0.92
PI 227333×M3	143	49	50	23	265	0.96	0.81
A.K. Harrow×M3	88	17	18	8	131	2.87	0.41
PI 261474×M1[b]	47	12	19	7	85	0.44	0.93
PI 427099×M1	101	43	34	8	186	1.91	0.59
PI 297544×M1	41	18	13	10	82	0.94	0.82
Total	644	217	196	72	1129	0.30	0.96

[a] Midwest Oilseeds M3=527–8×91133; F_1 plants are heterozygous, Ms ms
[b] Midwest Oilseeds M1=502–19×82854; F_1 plants are heterozygous, Ms ms

Table 6 F_2 segregation for fertility/sterility from crosses between known mutations at *ms1*, *ms2*, *ms3*, *ms4*, *ms5*, and *ms6* loci with ms soybean. 3:1=3 fertile:1 sterile; 9:7=9 fertile:7 sterile

Cross combinations	Number of families		χ^2 (1:1)	*P* (*df*=1)
	(3:1)	(9:7)		
ms1 ms1×M3[a]	6	9	0.60	0.44
ms2 ms2×M3	4	4	0.00	1.00
ms3 ms3×M3	11	2	6.20	0.01
ms4 ms4×M3	1	7	4.50	0.03
ms5 ms5×M3	6	3	1.00	0.32
ms6 ms6×M3	10	11	0.05	0.82

[a] Midwest Oilseeds M3=527–8×91133; F_1 plants are heterozygous, Ms ms

or 9:7 ratios (Tables 6 and 7). These data indicate that the male sterility of ms was controlled monogenically by a single recessive allele and was, therefore, different from the six known soybean male-sterile loci. Regarding *ms3* and *ms4*, there were two segregation patterns in the F_2 generation. With *ms3*, 11 families segregated in a 3:1 ratio and two families in a 9:7 ratio. Classification of F_3 plants descended from fertile F_2 (9:7) families of *ms3* cross-combination confirmed the F_2 (9:7) ratio (Table 8). With *ms4*, one family segregated in a 3:1 ratio and 7 families segregated in a 9:7 ratio. Similarly, classification of F_3 plants descended from the single F_2 (3:1) family of the *ms4* cross-combination confirmed the F_2 (3:1) ratio (Table 8).

Table 7 F_2 segregation for fertility/sterility from crosses between known mutations at *ms1*, *ms2*, *ms3*, *ms4*, *ms5*, and *ms6* loci with ms soybean

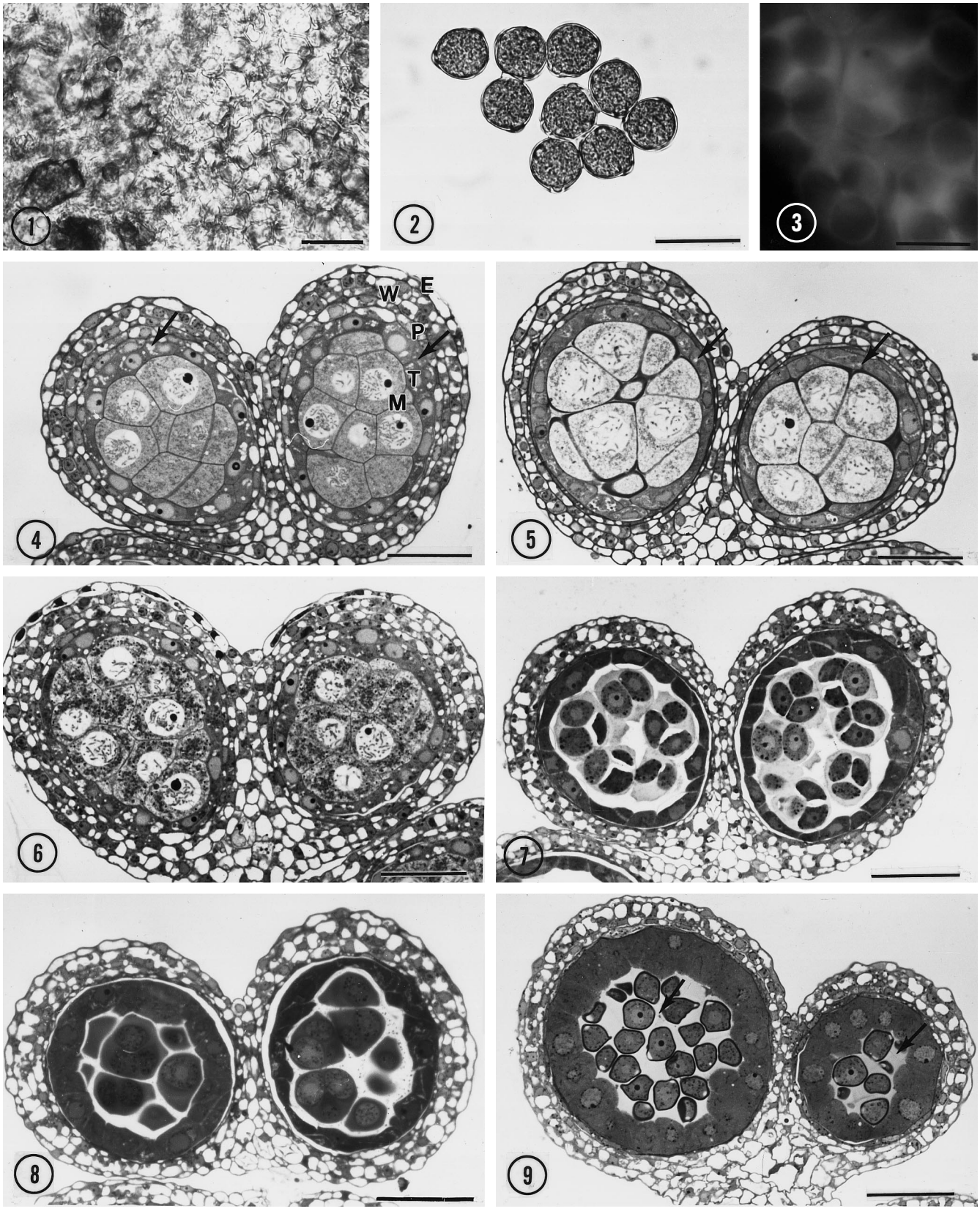
Cross combinations	Number of plants		χ^2 (3:1)	*df*	*P*	Number of plants		χ^2 (9:7)	*df*	*P*
	Fertile	Sterile				Fertile	Sterile			
ms1 ms1×M3[a]										
Total	561	200	1.30	5	0.93	651	530	2.16	8	0.98
Pooled			0.66	1	0.42			0.61	1	0.43
Homogeneity			0.64	4	0.96			1.55	7	0.98
ms2 ms2×M3										
Total	257	100	1.73	3	0.63	222	182	2.12	3	0.55
Pooled			1.72	1	0.19			0.28	1	0.60
Homogeneity			0.01	2	1.00			1.84	2	0.40
ms3 ms3×M3										
Total	728	255	2.77	10	0.99	75	56	0.06	1	0.81
Pooled			0.46	1	0.50			0.07	1	0.80
Homogeneity			2.31	9	0.99				0	
ms4 ms4×M3										
Total	28	6		0		261	217	1.65	6	0.95
Pooled			0					0.53	1	0.80
Homogeneity			0					1.12	5	0.95
ms5 ms5×M3										
Total	260	94	1.64	5	0.90	69	49	0.53	2	0.77
Pooled			0.46	1	0.50			0.23	1	0.63
Homogeneity			1.18	4	0.88			0.30	1	0.58
ms6 ms6×M3										
Total	441	141	2.94	9	0.97	543	445	4.05	10	0.95
Pooled			0.19	1	0.66			0.67	1	0.41
Homogeneity			2.75	8	0.95			3.38	9	0.95

[a] Midwest Oilseeds M3=527–8×91133; F_1 plants are heterozygous, Ms ms

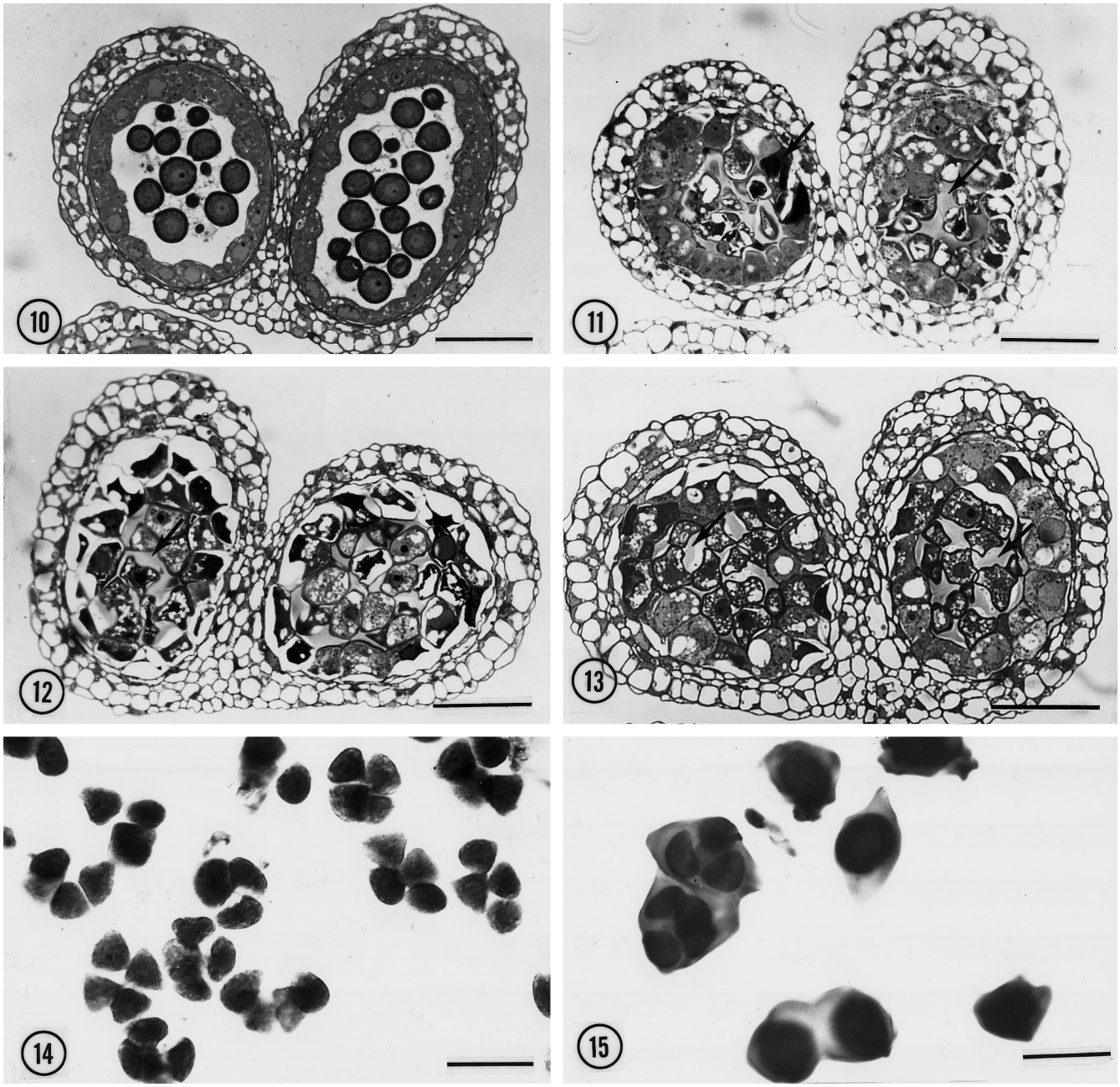
Table 8 F_3 segregation for fertility/sterility from crosses between known mutations at *ms3* and *ms4* loci with ms soybean. Male-fertile F_2 plants single-plant threshed from *ms3* families segregating 9:7 and from *ms4* family segregating 3:1

Cross combinations	No. plants		χ^2 (9:7)	*df*	*P*	No. plants		χ^2 (3:1)	d*f*	*P*
	Fertile	Sterile				Fertile	Sterile			
ms3 ms3×M3[a]	448	353								
Total			2.90	4	0.57					
Pooled			0.03	1	0.86					
Homogeneity			2.87	3	0.41					
ms4 ms4×M3						1213	395			
Total								5.16	9	0.82
Pooled								0.16	1	0.69
Homogeneity								5.00	8	0.76

[a] Midwest Oilseeds M3=527–8×91133; F_1 plants are heterozygous, Ms ms



Figs. 1–9 Squashes and cross-sectional images of male-fertile and male-sterile soybean anthers. All *bars* equal 10 μm. **Fig. 1** Squash of mature anther from male-sterile plant in I_2KI showing degenerated microspores. **Fig. 2** Squash of mature male-fertile anther in I_2KI showing engorged pollen grains. **Fig. 3** Squash of male-sterile anther in aniline blue at late microspore or early pollen stage showing microspores still encased in callose. **Fig. 4** Fertile sporogenous mass stage. Epidermis (*E*), wall layers (*W*), parietal layers (*P*), tapetum (*T*), and male cells (*M*). **Fig. 5** Male-sterile meiocyte anther in prophase of meiosis. **Fig. 6** Male-fertile meiocytes in prophase of meiosis. **Fig. 7** Male-fertile microspore tetrads. **Fig. 8** Male-sterile microspore tetrads. **Fig. 9** Late microspore tetrads from male-sterile plant. Tapetal cells enlarged, tetrads appear normal



Figs. 10–15 Cross-sectional images of male-fertile and male-sterile soybean anthers. All *bars* equal 10 μm. **Fig. 10** Male-fertile young microspores after dissolution of callose. **Figs. 11–13** Male-sterile post-tetrad stages showing degenerated tetrads still surrounded by callose (*arrows*). Tapetum has degenerated and contains unidentified densely-stained material. **Fig. 14** Isolated sterile tetrads treated with crude extract from fertile anthers. Callose is absent, stain is lacmoid. **Fig. 15** Isolated normal tetrads treated with crude extract from sterile anthers. Callose still surrounds the tetrads, stain is lacmoid

Cytology

Anther and pollen development in male-fertile soybean has been described by Carlson and Lersten (1987), Albertsen and Palmer (1979), and Buntman and Horner (1983). Observations of male-fertile development are, therefore, presented only where pertinent. Anthers from male-sterile plants were white, instead of the yellow color typical of fertile anthers. Also, anthers of male-sterile plants were slightly smaller at maturity than the anthers of fertile plants. When mature anthers were squashed in I_2KI solution, male-sterile anthers consisted mostly of degenerated microspores (Fig. 1), whereas densely staining pollen grains were observed in fertile anthers (Fig. 2). Aniline blue staining indicated that callose was retained around degenerated microspores in male-sterile anthers (Fig. 3).

Anther development in male-sterile plants appeared normal during the earliest stages of microsporogenesis. Each of the four young locules contained sporogenous

mass cells (SMCs) which were surrounded by an epidermis, endothelium, and up to two parietal layers. The innermost layer, the tapetum, separated the SMCs from the parietal layers (Fig. 4, arrows). Microspore mother cells (meiocytes) differentiated from SMCs.

During prophase I, small cytoplasmic vacuoles appeared in the tapetal cells of both male-sterile (Fig. 5, arrows) and male-fertile anthers (Fig. 6). As meiosis progressed, meiocytes divided to form dyads, and then tetrads, of microspores in both male-fertile (Fig. 7) and male-sterile (Fig. 8) anthers. Microspores within tetrads became isolated by callose (Figs. 7, 8). The tapetal cells in male-sterile anthers enlarged, and became densely stained (Figs. 8, 9). The tapetal cells of male-fertile anthers remained cytoplasmically dense. The sheath of callose surrounding the tetrads from male-sterile anthers remained (Figs. 9, arrows, 11–13), and each microspore cytoplasm became highly vacuolate (Figs. 11–13). In male-fertile anthers, callose was degraded, and individual microspores were released (Fig. 10).

The behavior of the tapetum in male-sterile anthers was variable. In some locules, male cells degenerated, whereas the tapetum retained its cytoplasm and seemed functional (Figs. 8, 9); or the tapetal cells became highly vacuolate (Fig. 12) or enlarged (Fig. 9). Some tapetal cells accumulated a densely staining material before collapsing (Fig. 11). In later stages of development, the tapetum collapsed into a mass of darkly staining material (Fig. 13).

Microsporogenesis in male-sterile anthers did not progress beyond the tetrad stage. While still enclosed in callose, many tetrads shriveled and collapsed (Fig. 11). The tapetal cell walls of both the male-fertile and male-sterile anthers remained intact (Figs. 10, 11), but there was little cytoplasm remaining in the male-sterile tapetal cells. When anthers were mature, there were only degenerated cells within the locules of male-sterile anthers (Fig. 12), whereas engorged pollen grains were present in the locules of male-fertile anthers (Fig. 2). Even at or near anthesis, microspores from male-sterile plants remained encased in callose (Fig. 13).

The results of tests for callase activity in both male-fertile and male-sterile anthers are shown in Figs. 14 and 15. Callose around isolated fertile and sterile tetrads (Fig. 14) was digested after treatment with fertile anther enzyme extracts but not after treatment with sterile anther extracts (Fig. 15, fertile tetrads). The results indicate there was no callase activity in male-sterile anthers but callase was active in the male-fertile anthers.

Discussion

The genetic data indicate that the male-sterile soybean (ms) in this study is genic male sterile (gms) and is controlled monogenically by a single recessive allele. The mutation occurs at a locus that differs from the already characterized *ms1*, *ms2*, *ms3*, *ms4*, *ms5*, and *ms6* soybean lines. A skewed ratio was observed in cross-combinations with *ms3* and *ms4*. This was the result of the small number of F_2 families tested because F_3 data confirmed F_2 data, rather than of gamete interaction between ms and *ms3* and *ms4*. Skorupska and Palmer (1990), however, observed a skewed ratio with *ms4* in test crosses (excess of fertile F_1 plants). The authors had no plausible explanation for the skewed ratio.

The co-segregation of a closely linked marker locus *(W1*) with a male-sterility locus (*ms6*) was used to produce large quantities of F_1 hybrid soybean seed (Lewers et al. 1996). The *W1* and *T1* loci were independent of the ms locus in the present study, which precludes their use in any soybean breeding program using the present ms line.

Based on results of the glasshouse experiment, this is a completely male-sterile line. Marrewijk (1969) reported that the phenotypic effect of partial male-sterility systems was subject to environmental modification. Temperature has more influence than any other environmental factor; however, water stress, photoperiod, nutrient supply, and hormone applications also influence male-sterile phenotypes (Heslop-Harrison 1957; Edwardson 1970). In soybean, the *msp* mutant is affected by temperature (Stelly and Palmer 1980; Carlson and Williams 1985). The expression of the male-sterile gene in this study is at least not affected by the summer glasshouse environment.

Compared to known male-sterile soybean mutants, this new gms mutant is similar to *ms2*, and more similar to *ms3* phenotypically, but genetically controlled by different genes. All three mutants result in a degeneration of tetrads because release of microspores from their encasing callose walls is prevented, a phenomenon also described in other nonlegume species. For example, the failure of callose to break down at the proper time in cms petunia anthers resulted in sterility (Frankel et al. 1969). The retention of callose seemingly blocks developmental metabolic processes (physical constraints are imposed by the callose wall) and intercellular communication between male cells and locular fluids and between male cells and surrounding tissues.

Abnormal behavior of callase has been observed in several male-sterile systems. Previous studies indicate that the enzyme callase is synthesized in the tapetum, then secreted into the locules, and degrades the callose walls surrounding the microspore tetrads. The timing of production and release of callase by the tapetum, therefore, seems to be critical for normal pollen development (Eschrich 1961; Frankel et al. 1969; Mepham and Lane 1969; Izhar and Frankel 1971; Stieglitz and Stern 1973; Worrall et al. 1992; Tsuchiya et al. 1995). Premature breakdown of callose was observed in male-sterile sorghum (Warmke and Overman 1972) and in cms petunia (Izhar and Frankel 1971). Worrall et al. (1992) and Tsuchiya et al. (1995) reported that premature breakdown of callose caused male sterility in transgenic tobacco. Absent or delayed callose degradation was reported in *ms2* (Graybosch and Palmer 1985) and *ms3* soybean (Buntman and Horner 1983), in cms *Capsicum* (Horner

and Rogers 1974), and in cms *Helianthus* (Horner 1977), and is similar to what is reported here for the ms mutant. Therefore, the timing of callase activity is critical for normal development of microspores.

As in many of the male-sterile mutations of angiosperms, abnormal tapetum activity or premature degeneration is associated with the abortion of microspores (Laser and Lersten 1972; Gottschalk and Kaul 1974; Koltunow et al. 1990). The most obvious abnormalities of tapetal cells in this soybean male-sterile mutant were cell enlargement, the accumulation of an unidentified, densely staining material, and premature degeneration. This accumulated material, based on its staining, is suspected to be sporopollenin or its precursors. The tapetum is regarded as the site for synthesis for precursors of sporopollenin (Echlin 1971; Horner and Pearson 1978; Nakashima et al. 1984).

Based on the results presented in this study, there are four possibilities for the underlying cause of the sterility in this new genic male-sterile line: (1) callase is not produced, or is produced below the threshold level to digest the existing callose; (2) the callase is molecularly defective; (3) the callose is molecularly defective and is not sensitive to the callase; and (4) the callase is not active in the environment of the locular fluid (i.e., suboptimal pH). One of these possibilites, namely, that the callose is molecularly defective, has been tested using the crude anther enzyme extracts, and is discounted as the cause of the sterility. Both the fertile- and sterile-tetrad callose walls were digested with the crude extracts from male-fertile anthers. This leaves the other three possibilities that are presently being tested by molecular approaches.

Genic male-sterile mutants have been proposed for many crop species breeding programs (Horner and Palmer 1995). Controlled production of hybrid seed is necessary for breeding programs and genetic studies. The most feasible methods should utilize close genetic linkage between a male-sterility locus and a seedling marker locus. In soybean, use of the close genetic linkage (2–4 cM, Skorupska and Palmer 1989) between a male-sterility locus and a seedling marker locus (*W1*) is known as the co-segregation method to produce F_1 seeds (Lewers et al. 1996). Development and use of this method will assist in the elucidation of the genetic control of complex traits, the identification of lines to improve these traits, the improvement of populations for these traits through the use of recurrent selection, and determination of the agronomic potential of commercial hybrid soybean. The identification of additional soybean genic male steriles linked to a seedling marker locus will reduce the genetic vulnerability of soybean production of a single genic male sterile. The new genic male-sterile line described in this study, because of its high seed set, would be valuable for breeding programs if a useful linked genetic marker or a molecular marker can be identified.

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